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Article type : The Scientific Naturalist

Running head: The Scientific Naturalist

Rediscovery of a presumed extinct species, *Salvelinus profundus*, after re-oligotrophication

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Manuscript received 6 December 2019; revised 29 January 2020; accepted 25 February 2020; final version received 30 March 2020.

Lake Constance (47° 38’ N, 9° 22’ E) is a deep (max. depth 251m) and large (surface area 536 km^2) postglacial lake in Central Europe. Originally, it harboured two charr species – *Salvelinus umbla* and *S. profundus*. The first is a medium-sized, colourful, winter spawning charr, which is widespread across Central European lakes, the second a small, pale, summer spawning, deepwater charr, which is endemic to Lake Constance (Schillinger 1901, Kottelat and Freyhof 2007). *S. profundus* has exceptionally large eyes and the upper jaw strongly overlaps the lower jaw. These traits are both considered adaptations to its life in the deep where it mostly feeds on profundal benthos (Schillinger 1901). During the second half of the last century, Lake Constance became eutrophic, resulting in oxygen-depletion in deep waters (IGKB 2004). The anoxic conditions harmed the development of eggs by profundal spawning fish (Baer et al. 2017). Thanks to strict management interventions, the lake has returned to an oligotrophic state, and oxygen is again available in the water column down to the greatest depth (IGKB 2004).

However, during peak eutrophication in the late 1970s, deepwater species such as the whitefish *Coregonus gutturosus* and the charr *S. profundus* disappeared (Freyhof and Kottelat 2008a,b). While both species were common and commercially harvested before eutrophication, intense targeted sampling over a decade after re-oligotrophication remained fruitless, and they were therefore declared extinct according to IUCN criteria (Freyhof and Kottelat 2008a,b). Today, *C. gutturosus* is considered a prime example for speciation reversal, whereby a change in environmental conditions, which are necessary to maintain reproductive isolation among evolutionarily young species, causes species to go extinct by collapsing into a hybrid swarm (Vonlanthen et al. 2012, Frei et al. unpublished data). The simultaneous disappearance of the ecologically similar *S. profundus* led to the conclusion that it shared the fate of *C. gutturosus* (Vonlanthen et al. 2012).

However, in 2012, a colleague, Jasminca Behrmann-Godel, informed us about a small, pale, summer spawning charr that she and others had caught. On several previous occasions, small individuals of *S. umbla* had wrongly been identified as *S. profundus* (Kottelat and Freyhof, 2007), so in this case, we were hopeful that it could be the presumably extinct species, since it showed several of its traits. In 2010, a large project (“Projet Lac”) started to assess the fish community for each of the large pre-

alpine lakes in and around Switzerland through habitat stratified random fishing (Alexander et al. 2015). We were therefore very much looking forward to autumn 2014, when it was Lake Constance's turn in that project (Alexander et al., 2016). Besides the standardized random fishing program, we also set two additional nets to target the extinct species, at the same location and depth where Dörfel had caught the last specimens in 1972. Surprisingly, we caught seven charr at this location that closely matched the description of *S. profundus* (Fig. 1A-C), and an additional one during the large fishing campaign (Fig. 1E). The other charr species, *S. umbla* (N=12) was caught across entire Lake Constance. At the historically known *S. profundus* hotspot, we even had the two distinct species in the same net. Nonetheless, *S. profundus* was found at deeper depths than *S. umbla* (median depth: 90m vs. 37m, Wilcoxon test $p<0.001$). The rediscovery of this ecologically unique species that has survived a major environmental perturbation has prompted us to reassess its phenotypic and genetic differentiation relative to its sympatric relative.

Our morphological data suggest that the rediscovered charr species differs considerably from *S. umbla* in many traits, including ecologically important ones, and that is has maintained most of these traits through the period of eutrophication. We measured 24 linear morphometric distances (Appendix S1: Fig. S1) on preserved charr caught in autumn 2014 (8 *S. profundus* and 11 *S. umbla*). The two species clearly differ in overall morphology, as revealed by a morphological PCA based on all traits (Fig. 2A). The rediscovered deepwater *S. profundus* specimens have significantly larger eyes, wider upper jaws at the front, and a more subterminal mouth than *S. umbla* individuals (Fig. 2B), all of which were characteristic for *S. profundus* before the eutrophication period (Schillinger 1901). Furthermore, they have longer anal fins, and marginally longer snout, longer maxilla, and shallower body depth at the anterior insertion of the anal fin (Appendix S1: Table S1). Colours also match historical descriptions: while *S. umbla* has green-brown body coloration with red-yellow spots and prominent white fin margins, *S. profundus* has a pale body with faint spots, and no or only faint white fin margins (Fig. 1; Schillinger 1901, Kottelat and Freyhof 2007).

However, the two species no longer differ in a previously distinguishing trait: namely *S. profundus*, which historically had very low gill raker numbers (median: *profundus* 22, *umbla* 28; Dörfel 1974) no

longer differs from that of *S. umbla* (Fig. 1C; *profundus* 23; *umbla* 23; Wilcoxon test $p = 1$). The two species converged in this trait, thereby both undergoing a significant (Wilcoxon test: *profundus* $p = 0.043$, *umbla* $p < 0.001$) and rapid phenotypic change (*profundus* -0.04 haldanes, $CI_{95} = -1.84$ to -0.002; *umbla*: 0.31 haldanes, $CI_{95} = 0.24$-0.38; generation times were estimated from average age of pre-eutrophication charr (Dörfel 1974)). These rates are comparable to those of Alpine whitefish during eutrophication (significant absolute rates of 0.07 - 0.59 haldanes, Bittner et al. 2010), and the pattern of convergence (Fig. 1C) is reminiscent of partial speciation reversal in Alpine whitefish (Vonlanthen et al. 2012). Interestingly, convergence was asymmetric in that *S. umbla* shifted strongly towards gill raker numbers of the historical *S. profundus*. Given the rate and magnitude of change, this shift was probably influenced by introgression and might be adaptive for feeding on more benthic prey.

At same age, *S. profundus* individuals were consistently smaller than *S. umbla* individuals (Fig. 2D). We obtained age data from otoliths immersed in water, read under a binocular microscope (two readings by CJD; if different, lower estimate was used). Growth models revealed greater asymptotic growth for *S. umbla* than for *S. profundus*. However, confidence intervals of asymptotic length overlapped (umbla $L_a = 383$mm, CI=301-464mm; profundus $L_a = 289$mm, CI = 112-467mm), potentially due to small sample sizes. Our results are consistent with the historically described smaller size and slower growth of *S. profundus* as compared to *S. umbla* (Schillinger 1901, Dörfel 1974).

We found significant genetic differentiation between the two sympatric species, suggesting reproductive isolation between them. For this study, tissue samples for genetic analyses were available for all 8 *S. profundus* and for 7 *S. umbla* individuals caught in September 2014. To increase sample size, we added 10 *S. umbla* samples obtained from spawning fisheries in November 2012. For all 25 samples, we extracted whole-genomic DNA using phenol-chloroform extraction and genotyped them at nine microsatellite loci as described in Doenz et al. (2019). The two species were significantly genetically differentiated at two of nine (Appendix S1: Table S2) and at all microsatellite loci combined (multilocus $F_{ST} = 0.058$, $p < 0.001$, Arlequin v. 3.11 with 1000 permutations, Excoffier and Lischer 2010). This multilocus F_{ST} value lies in the range of other sympatric charr species (Moccetti

et al. 2019, Doenz et al. 2019). In line with genetic differentiation, the two species occupied different areas in genetic PC1-2 space (Fig. 2E).

Before the eutrophication period, resource polymorphism within *S. profundus* was described: some individuals fed at great depths on benthic invertebrates, others in the pelagic on a now extinct zooplankton species (Elster, 1936). Polymorphism in *S. profundus* is also supported by our morphological and genetic data: The seven *S. profundus* from the historically known location match the description of *S. profundus* better than the individual caught far away (Fig. 1). Furthermore, one of the seven individuals from the *S. profundus* hotspot was large and had extremely long teeth, both of which are typical for piscivorous charr (Fig. 1D). Upon exclusion of these two atypical *S. profundus*, genetic differentiation between *S. umbla* and *S. profundus* increased (multilocus $F_{ST} = 0.086$, $p < 0.001$; also at most individual loci, Appendix S1: Table S2). Accordingly, in genetic PC1-2 space, these two individuals were at the boundary of the two species (Fig. 2E). This is consistent with recent introgression between *S. profundus* and *S. umbla*, or with the existence of more than one profundal charr species in Lake Constance, as it is known from charr radiations elsewhere in the world (Knudsen et al. 2016, Moccetti et al. 2019).

The rediscovery of the endemic deepwater charr of Lake Constance is great news at a time where endemic species diversity of lacustrine fish is rapidly eroding (WWF 2018). Strong management interventions recovered an oligotrophic state of Lake Constance, thereby contributing to rescuing *S. profundus*. Given that many morphological traits of the historical *S. profundus* are still present, its unique ecological functions have likely survived eutrophication. A major question arising is: what allowed this species to survive eutrophication while its ecological analogue in the genus *Coregonus* in Lake Constance went extinct? One could hypothesize that the sympatrically evolved whitefish species (Hudson et al. 2010) are younger than the charr species. Furthermore, spawning ecology or genomic architecture of differentiation may differ, whereby whitefish may have a more environment-dependent reproductive isolation which may also be more polygenic in architecture. Any of these factors or their combination would make the persistence of the profundal whitefish species more sensitive to environmental change and hybridization. Combining ecological and genomic data could help to

elucidate this question in the future. Finally, this rediscovery also showcases that the profundal habitat in large lakes is still one of the least explored environments that deserves our attention in terms of research and conservation even in Central Europe.

Acknowledgments

We thank the Projet Lac team for their help collecting the fish; the natural history museum of Bern, especially Lukas Rüber, for specimen curation; Corinne Schmid for assisting with the genotyping; Jasminca Behrmann-Godel for making us aware of this unusual specimen, and the fish ecology and evolution group of University of Bern and Eawag for discussions. Special thanks to David Frei for his insightful feedback.

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Data Availability

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.0zpc866v2

Figure 1. A) Drawing of the deepwater charr species *S. profundus* from Vogt and Hofer, 1909. B,C) Male and female *S. profundus* caught in 2014 at the historically known *S. profundus* site. D) *S. umbla* caught together with B,C). E) *S. profundus* caught far away from the historically known *S. profundus* site. F) A piscivorous looking *S. profundus* caught together with B,C).

Figure 2. The rediscovered profundal charr species *S. profundus* (darkblue) differs in morphology and genetics from its sympatric relative *S. umbla* (orange). A) The two species are distinguishable in morphospace of a PCA based on 24 linear morphometric distances (centred and normalized). Each trait was size-corrected by taking residuals from a pooled regression of log(trait) against log(standard length). B) Like before the eutrophication period (Schillinger, 1901), *S. profundus* has a stronger overlap of the upper jaw, larger eyes (size corrected), and wider upper jaws (size corrected) than the sympatric *S. umbla*. C) The two species do not differ in gill raker numbers anymore (counted on the first left gill arch). D) *S. profundus* shows smaller asymptotic growth than *S. umbla*. We fitted von Bertalanffy growth models (solid lines) using the function "growthmultifit" of the R-package "fishmethods", assuming group-specific asymptotic length (Nelson, 2018). Sample size was too small to estimate group specific growth coefficients. E) *S. profundus* and *S. umbla* are separated in genetic (9 microsatellite loci) PC1-2 space, while the two atypical *S. profundus* lie at the boundary of the two groups (light blue asterix: Fig. 1E; blue asterix: Fig. 1F). We performed the genetic PCA using the "dudi.pca" function of the R-package "adegenet" (Jombart, 2008) with default settings (centering and scaling the data). Missing data were replaced by mean allele frequencies. Generally, all analyses were performed in R v.3.6.0 (R Core Team, 2019), if not stated differently.